Molecular Dissection of the Hematopoietic Stem Cell Niche Using Laser Capture Microdissection (LCM) Technology in the Developing Murine Bone Marrow

Jingcheng (Jason) Wang[1], Younghun Jung[1], Jianhua Wang[1], Aaron Havens[1], Yanxi Sun[1], Taocong Jin[2], and Russell S. Taichman[1]

[1]Department of Periodontics, Prevention, and Geriatrics, University of Michigan School of Dentistry, Ann Arbor, MI
[2]Department of Cariology, Restorative Sciences, and Endodontics, University of Michigan School of Dentistry, Ann Arbor, MI

Several reports suggest that increasing the number of osteoblasts (OBs) increases the number of hematopoietic stem cells (HSCs) in the marrow. Yet the molecular mechanisms for these observations are unclear. The predominant hypothesis is that the differences reflect more stem cell niches. Yet what factors regulate the stem cell niche are poorly understood. Laser capture microdissection (LCM) techniques have been developed to permit the rapid isolation of pure populations of cells from heterogeneous tissues. We used LCM to recover RNA from endosteal osteoblasts (which support hematopoiesis) versus periosteal OBs (which do not) and evaluated the differences in expression levels of several well known hematopoietic-supportive molecules and for a select group of proteins. Protocols and instrumentation setups were performed following the recommendations from Affymetrix Co. (Santa Clara, CA) by the University of Michigan Dental School Microarray Facility. This included the synthesis of biotin-labeled cRNA and hybridizations to the GeneChip® Mouse Expression 430A arrays. The data was initially analyzed using Affymetrix GeneChip related software (GCOS v1.1.1 and Data Mining Tool). Later, average gene intensity values were determined using a software package (DNA-Chip (dChip version 1.1) in consultation with the University of Michigan Cancer Center Microarray Core Facility. Based upon these analyses, several interesting candidate molecules have emerged which are significantly expressed in endosteal OBs (which support hematopoiesis) relative to periosteal OBs (which do not).

Among the genes differentially expressed by endosteal OBs are *SDF-1*, *annexin II*, *S100A10* (p11, the annexin II binding partner), *Vcam-1, CD164*, and a *Notch-1* homologue. As our samples were taken from newborn animals in regions where the bones were growing radially, many genes involved in matrix synthesis were expressed to a greater degree by periosteal OBs. We are currently pursuing studies to determine the function of several of novel molecules. For example, stromal interaction molecule 1 (STIM1) is a cell surface transmembrane glycoprotein implicated in the control of tumor growth and stromal-hematopoietic interactions. The murine *Ym1* gene belongs to a family of mammalian genes homologous to the chitinases from lower organisms, such as insects. Ym1 is a secretary protein transiently produced by activated peritoneal macrophages such that the temporal and spatial expression in myeloid precursors suggests Ym1 may be involved in early hematopoiesis and inflammation. Thus far we have confirmed that *SDF-1, stim1 and Ym1* are expressed differentially by endosteal osteoblasts by *in situ* hybridization or immunohistochemistry. In addition we

demonstrate that antibody to annexin-II, S100A10, jagged-1 (a notch ligand), N-cadherin, and CD164 also regulate the binding of blood cells to OBs. This data demonstrate the utility of LCM and demonstrate that we have identified novel hematopoietic targets for further investigation.